Consistency of Developmental Effects of Nickel and Nickel Compound in Human Studies: Associations Between Airborne Nickel Exposure and Low Birth Weight

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Executive Summary

A review of the population-based studies of low birth weight (LBW) associated with nickel air exposures indicates that they suffer from limitations derived from their reliance on single pollutant models to assess risks in multi-pollutant studies. While these studies are useful to generate hypotheses, they are not robust enough to establish reliable evidence of causality. In fact, a comparison of the LBW results from Ebisu and Bell (2012) to those in the refinery workers' study of Vaktskjold et al. (2007) demonstrate that the risks predicted from the population study were not realized in workers with daily air nickel absorption levels 60 to 376-fold higher than the minimal needed to detect these effects with sufficient statistical power.

The absence of the population-based study's predicted effects in the workers' study provides contradictory evidence for a causal association between LBW and nickel exposure and underscores the essentiality of testing hypotheses generated by individual pollutant analyses in multi-pollutant studies. The evidence in support of listing nickel and nickel compounds as developmental toxicants under Prop65 is not "sufficient" or even "limited" since there are negative studies that "*have sufficient power to call into question the repeatability of the observation in the positive study (CalOEHHA, 1993)*"

Introduction

DARTIC will be discussing the human and animal evidence for developmental and reproductive toxicity of nickel and nickel compounds at their October 10th meeting. I have reviewed the epidemiological evidence for developmental effects of nickel exposure focusing on the statistical analyses of low birth weight in general population studies using single pollutant models and the consistency of the associations between low birth weight and nickel air exposure across studies.

The studies listed in the OEHHA doc are based on two very different populations: the general public exposed to nanograms Ni/m^3 as PM2.5 or PM10, and workers exposed to hundreds of micrograms of Ni/m^3 as inhalable aerosols. However, because the aerosols have different particle sizes and can result in different deposition in the respiratory tract and absorption after inhalation, differences in the internal doses were considered.

Results

<u>Statistical analyses of low birth weight in general population studies</u>.

All of the general population-based air pollution studies considered by OEHHA doc (Bell et al, 2010; Ebisu and Bell, 2012; Basu et al., 2014; Laurent et al., 2014; Pedersen et al., 2016) rely primarily on

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statistical analysis of low birth weight outcomes in single pollutant (univariate) models that inadequately take into account the effects of the multi-pollutant mixtures to which the study populations were exposed.

At best, these studies suggest possible causal associations between individual pollutant exposures and gestational outcomes, but the correlations between different pollutant exposures make inferences with respect to any individual pollutant (based on univariate modeling) highly uncertain. Two studies attempted to address this problem by using models with two pollutants (Ebisu and Bell, 2014; Pedersen et al., 2016). In Pedersen et al. (2016), the effect of nickel exposure (marginally significant in a univariate model; OR=1.14; 95% CI = 1.00-1.29) was not statistically significant after adjustment for the effects of either PM2.5 or PM10 mass. This study did not attempt to adjust particulate effects for those of gaseous pollutant exposures (e.g. NO_2, O_3, etc.).

Ebisu and Bell (2014) attempted to address possible gaseous pollutant effects, but only incompletely. In their study, they provided the results of two pollutant modeling, estimating risks for individual pollutants after adjustment for exposure to other pollutants (only when correlations between the pairs were less than 0.5). Nickel exposure was highly correlated with that of elemental carbon (EC) (correlation of 0.49), as well as NO_2 (correlation of 0.72) After adjustment for the effect of EC, the estimated percent change in relative risk of low birth weight (LBW) per interquartile range (IQR) of nickel dropped by a factor of 2, with a confidence interval whose lower limit was very near zero. It is interesting that the researchers did not adjust for the effect of NO_2 on nickel-related risk (which appeared to be large based on univariate analysis); had NO_2 been included in a two pollutant model (as with EC), it is likely that similar, if not larger, reduction in the estimated risk associated with nickel would have occurred.

The point of the above observation is not to reject the Ebisu and Bell study for neglecting to include an analysis adjusting for the effects of NO_2 exposure (likely because of the instability of the resulting regression coefficients), but rather to point out the deficiencies associated with modeling the effects of individual pollutants without regard for the effects of other pollutants. Single pollutant modeling may be productive from the perspective of narrowing the field of hypotheses for further investigation, but it gives incomplete and possibly misleading information regarding causality for exposure to individual pollutants.

Consistency of the associations between LBW and nickel exposure across studies.

As mentioned above, there are several general population-based air pollution studies that have conducted statistical analysis of low birth weight outcomes and air exposure to nickel (Bell et al, 2010; Ebisu and Bell, 2012; Basu et al., 2014; Laurent et al., 2014; Pedersen et al., 2016). Some of the studies are quite large (e.g., n>1,000,00 for the Ebisu and Bell).

To test the hypothesis that airborne nickel exposure has gestational effects on birth weight, a study that more closely mimics a controlled experiment was conducted by Vaktskjold et al. (2007). In it, the incidence of LBW in infants in female nickel workers was compared to that of a background population in the same geographic location. The study provides an opportunity to ascertain whether the predicted changes in LBW incidence at small increases of airborne nickel exposure (for example, shown by Ebisu and Bell) should have been seen at much higher differentials in levels of nickel exposure. There was, in fact, no evidence for LBW associated with nickel exposure in either Low exposure (OR=0.62; 95 CI=0.49-

0.79) or High exposure workers (0.79, 95 CI= 0.68-0.91) relative to the background (control) population. The power calculations are shown below.

1. Assessment of exposures to nickel. To answer the question of whether the absence of any of evidence of increased risks in the Vaktskjold et al. (2007) study relates to study power (n=1,242 for the high exposure group versus n>1,000,00 for the Ebisu and Bell study population), we examined external and internal exposures in the Ebisu and Bell (2012) versus the Vaktskjold et al. (2007) studies.

The first step involved assessing the workers' air exposures in the Low and High exposure categories in the study by Vaktskjold et al. (2007), as well as estimates of internal doses. The workers' exposure information was presented in Vaktskjold et al. (2006) (see Table 1 below).

Table 1. Air concentration values (from Vaktskjold et al., 2006 unless otherwise noted).

	GM inhalable soluble Ni μg Ni/m³	**Upper 95% CI inhalable soluble Ni μg Ni/m³**
Ambient air		
Typical value[1]	0.010 as PM2.5 or PM10	
Low Refinery exposure		
-copper refining	14 (22)[2]	17 (29)[2]
-Cu pyrometallurgical	14 (51)[2]	18(87)[2]
-matte convert	24	46
-beneficiation	3	5
-ore roasting	18	24
-ore smelting	62	92
-matte separation	84	120
Mean values	**31.3**	46
High Refinery exposure		
-anode casting old	250 (10,000)[3]	350
-anode casting new	150 (1500)[3]	240
-electrorefining old	210 (250)[3]	260
-electrorefining new	225 (280)[3]	350
-matte roasting	330 (11,000)[3]	450
-Ni carbonyl	17	26
Mean values	**197**	279

1. Soluble and oxidic nickel (e.g., Galbreath et al., 2003).
2. Values in parenthesis from Thomassen et al (2004) for inhalable "all Ni" among women.
3. Values in parenthesis from Thomassen et al (1999) for inhalable "all Ni".

To obtain a common exposure metric for the general population and workers, NiPERA calculated the systemic (internal) absorbed daily dose obtained from the air, which depends on the respiration rate, hours of exposure, deposition fractions, and absorption in different regions of the respiratory tract. These calculations are described in the Supplemental section for general population and Low and High exposure workers' groups For workers, the daily dose from inhalation in the low and high exposure workers are 15 μg Ni/day and 94 μg Ni/day, respectively. For the general population, the relationship between air and internal dose is shown below:

Absorbed dose from air (μg Ni/day) =1.9*airborne concentration (μg Ni/m^3)

2. Assessment of predicted risks of LBW.

Ebisu and Bell indicate a 5.7% change in the odds of LBW (defined as <2,500 g) for each interquartile range increment in nickel dose (0.007 μg Ni/m^3). The overall LBW rate in their study population is 2.8% (Odds=2.88%). LBW in the Russian study population was defined differently (below the 10th percentile of infant body weights), with a background rate of 9.4 % (Odds = 10.4%). The lower incidence of LBW for the Ebisu and Bell is understandable, in that they excluded births prior to 37 weeks, while Vaktskjold et al. examined LBW in premature as well as full term infants relative to the 10th percentile at each gestational week (28-45 weeks).

The results of Ebisu and Bell indicate a predicted incremental risk in LBW odds as:

$$Odds(exposure) = Odds_b * 1.057^n — Odds_b$$

where:

exposure = n * 0.007 μg Ni/m^3

n=the number of interquartile ranges

$Odds_b$=background Odds.

In applying this formula, I used the 2.88% background odds from the Ebisu and Bell study rather than the 10.4% background odds from the Vaktskjold et al. study. This was done to reflect the scale of the Ebisu and Bell statistical risk model in the range of LBW incidence data from which it was derived (which differs from that of the Vatkksjold et al. data because of the differing LBW metrics). It is a conservative approach, as it produces smaller estimates of incremental risk per unit dose (and lower statistical power to detect these risks) than would be obtained using the Vatkksjold et al. background odds.

I then calculated the power to detect Odds ratio differences predicted by Ebisu and Bell at these increased magnitudes of exposure in the background population (with conversion to absorbed dose), using the sample sizes of the background (n=19,921) and High (n=1,242) exposure populations in the Vaktskjold et al. (2007) study (n= 1,672 for the Low exposure group). Examples of these calculations for increased airborne exposure of up to 0.028 μg Ni/m^3 are shown in Table 2 below:

Table 2. Statistical Power to Detect Changes in Low Birth Weight Odds Ratio in Vaktskjold et al. (2007) High Exposure Group Relative to Odds Increases Predicted at Increased Nickel Exposure (Ebisu and Bell, 2012).

Background LBW rate[1]	Background LBW Odds	Incremental Exposure Concentration (μg Ni/m^3)	Incremental Absorbed Dose (μg Ni/day)	Increase in LBW Odds	LBW Odds with Exposure	Odds Ratio (Exposed/ Background)	Power to Detect Change in Odds Ratio[2]
0.094	0.104	0.000	0.000	0.000	0.104	1.00	0.050
0.094	0.104	0.007	0.013	0.002	0.105	1.02	0.054
0.094	0.104	0.014	0.027	0.003	0.107	1.04	0.067
0.094	0.104	0.021	0.040	0.005	0.109	1.06	0.090
0.094	0.104	0.028	0.053	0.007	0.111	1.08	0.126

[1] Vaktskjold et al. (2007)

[2] α=0.05; two-tailed testing

The results of the complete power analysis, using conventional levels of significance in statistical testing (α=0.05 and α=0.01; two-tailed testing) are shown in Figure 1.

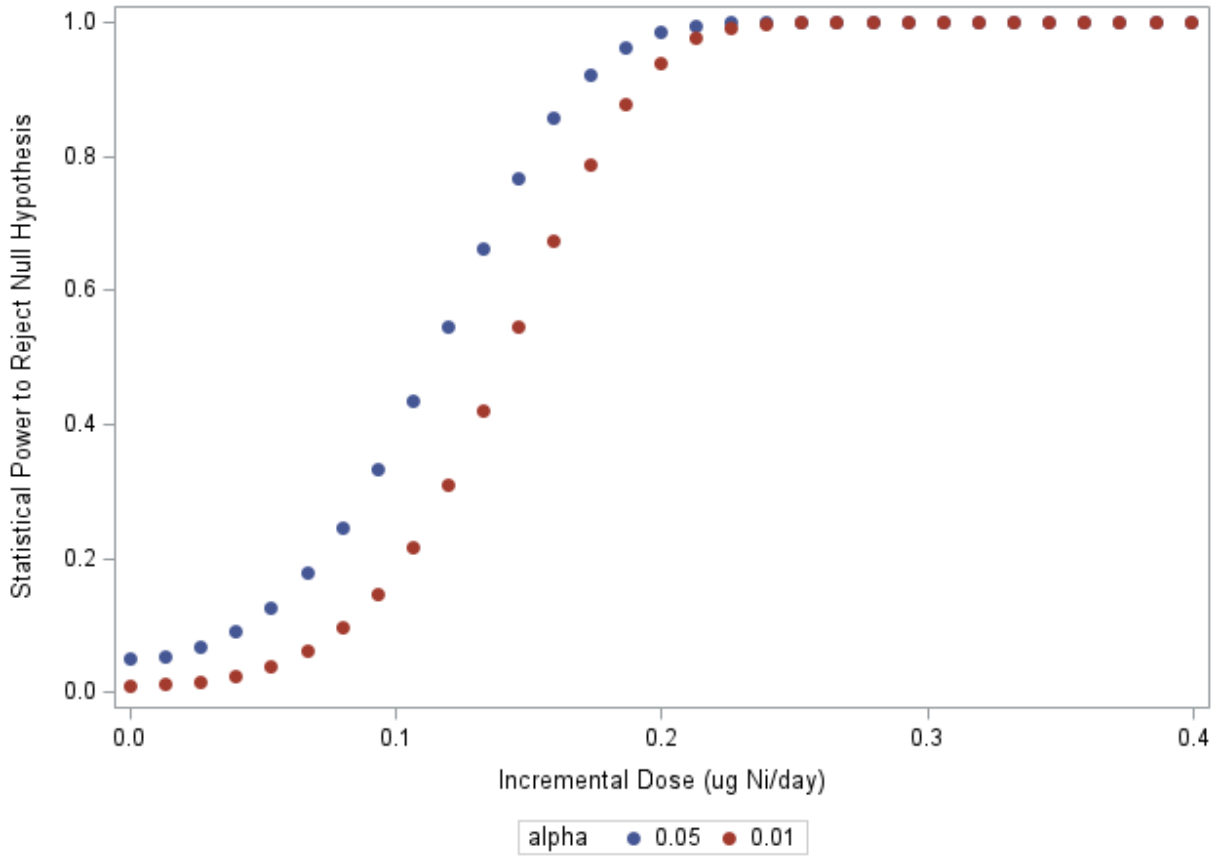

Figure 1. Statistical Power Analysis for Vaktskjold et al. (2007) Study Groups to Detect Effects Predicted by Ebisu and Bell (2012).

The smallest doses at which there is high statistical power to detect the predicted changes in LBW (e.g. 0.20 -0.25 µg Ni/day) fall well below the estimated internal doses associated with air exposure in the low exposure group (15 µg Ni/m^3) or high exposure group (94 µg Ni/m^3).

Conclusions

The effects predicted by Ebisu and Bell (2012) would be detected with virtual certainty in the groups of nickel workers exposed to 60 to 376-fold higher daily air nickel intake levels than the minimal needed to detect these effects with sufficient statistical power. The fact that the workers' study did not show these effects is contradictory to the hypothesis of a causal association between LBW and nickel exposure advanced by Ebisu and Bell (2012) and Bell et al. (2010), and underscores the essentiality of testing hypotheses generated by single pollutant analyses in multi-pollutant studies. The evidence in support of listing nickel and nickel compounds as developmental toxicants under Prop65 is not "sufficient" or even "limited" since there are negative studies that "*have sufficient powder to call into question the repeatability of the observation in the positive study (CalOEHHA, 1993)*".

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Supplemental analyses (conducted by A. Oller and M. Taylor of NiPERA, Inc).

To estimate the daily systemically absorbed dose from nickel in air we considered:

1) The fraction of nickel aerosol that will deposit in the trachea-bronchial (TB) and head regions of the respiratory tract and multiplied this value by the oral absorption value (range of values: 5% with food, 30% with fasting from De Brouwere et al., 2012). [The larger particles deposited in these regions will be swallowed and absorbed from the gastrointestinal tract. For workers, we assume 10% oral absorption for inhaled particles that are swallowed; for public we assume 30%.]
2) The fraction of nickel aerosol deposited in the pulmonary region of the respiratory tract and multiplied it by 50% (workers) or 100% (public) to calculate the direct lung absorption of nickel. [Different absorption values (within reported ranges) used for workers and public to present a most conservative comparison where workers' internal exposure is at low end of possible and public's internal exposure is at high end.]
3) Different exposure duration for workers (8 hours or 480 min) and for public (24 hours or 1440 min)
4) Different minute ventilation for workers (20 breaths/min) and for public (12 breaths/min)
5) Different tidal volume for workers (1024 cm^3/breath) and for public (625 cm^3/breath)

To calculate workers' deposition, we considered the particles size distribution of workplace soluble Ni aerosols reported in Oller et al. (2014) (Table A1) since these operations are similar to those in the Vaktskjold et al. (2007) study and use the density of Ni sulfate and the latest version of the Multiple Particle Pathway Deposition Model MPPD model to calculate the deposition fractions (MPPD v3.04). The respiratory tract deposition fraction in Head + TB was ~ 0.44 for electrolysis, 0.57 for matte grinding, 0.31 for roasting. The pulmonary deposition fraction was ~ 0.011 for electrolysis, 0.017 for matte grinding, and 0.008 for roasting. From these numbers and to be conservative we selected representative but low deposition fractions: 0.44 for TB + head and 0.01 for pulmonary. The calculations of daily deposited doses are shown below.

Low exposure: 31 µg Ni/m^3

Deposition = (Resp x Time x TV x Depfrac x Conc) =
Deposition TB + H = 20 breaths/min x 480 min x 1024 cm^3/breath x 0.44 x 31 µg Ni/1000000 cm^3 = 134 µg Ni
Deposition P = 20 breaths/min x 480 min x 1024 cm^3/breath x 0.01 x 31 µg Ni/1000000 cm^3 = 3.0 µg Ni

Selecting the most conservative (lowest) absorption values (10% oral and 50% inhalation), the absorption from inhalable workplace aerosol= (134 µg Ni x 0.1) + (3.0 µg Ni x 0.5) = (13.4 + 1.5) µg Ni = **15 µg Ni/day**

High exposure: 197 µg Ni/m^3

Deposition TB + H = 20 breaths/min x 480 min x 1024 cm^3/breath x 0.44 x 197 µg Ni/1000000 cm^3 = 852 µg Ni
Deposition P = 20 breaths/min x 480 min x 1024 cm^3/breath x 0.01 x 197 µg Ni/1000000 cm^3 = 19 µg Ni

Selecting the most conservative (lowest) absorption values (10% oral and 50% inhalation), the absorption from inhalable workplace aerosol: (852 µg Ni x 0.1) + (19 µg Ni x 0.5) = (85.2 + 8.5) µg Ni = **94 µg Ni/day**

For the purposes of comparing internal exposures in public to those of workers, we selected 10 ng Ni/m^3 (PM10, PM2.5) as a representative exposure value based on studies reported by OEHHA. To calculate general public deposition, we considered data on particles size distribution of ambient air (PM10) nickel aerosols (MMAD < 1 um) reported in Oller et al. (2014) (Table A2, based on particles size distribution of aerosols reported in Berico et al., 1997 for 3 periods of low, medium and high PM10) and used the density of Ni sulfate. Head + TB respiratory tract deposition fractions were ~ 0.12 Low, 0.18 Medium, 0.19 High. Pulmonary deposition fractions were ~ 0.121 Low, 0.117 Medium, 0.114 High. From these numbers and to be conservative we selected representative but high deposition fractions: 0.19 for TB + head and 0.12 for pulmonary.

Ambient air: 10 ng Ni/m^3

> Deposition TB + head = 12 breaths/min x 1440 min x 625 cm^3/breath x 0.19 x 10 ng/1000000 cm3 = 21 ng Ni
> Deposition P = 12 breaths/min x 1440 min x 625 cm^3/breath x 0.12 x 10 ng/1000000 cm3 = 13 ng Ni

Selecting the most conservative (highest) absorption values (30% oral and 100% inhalation), the absorption from ambient air aerosol= (21 ng Ni x 0.3) + (13 ng Ni x 1.0) = (6.3 + 13) ng Ni = **19 ng Ni/day**

For workers, the daily dose from inhalation of soluble nickel is conservatively considered, even if they also have exposures to insoluble nickel compounds and metal (lower bioavailability). For the general population, while exposures are to oxidic and soluble nickel compounds (Ni sulfate), these exposures are conservatively considered to be to the most bioavailable of the forms (Ni sulfate).

References cited in Supplemental analyses that are not included in OEHHA doc

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